MycoLight™ vPCR Star

Catalog number: 24211
Unit size: 1 mg

Component	Storage	Amount (Cat No. 24211)
MycoLight™ vPCR Star	Freeze (< -15 °C), Minimize light exposure	1 mg

OVERVIEW

MycoLight™ vPCR Star is the first commercial non-light-activated reagent that can be used to eliminate the false signal caused by dead cells in the PCR-based detection of viable bacterial and viral samples. It is effective and extremely convenient to use. Unlike Propidium monoazide (PMA), its activity does not require UV-light activation. MycoLight™ vPCR Star only penetrates cells with compromised cell membranes (non-viable cells) and reacts with DNA, rendering the DNA of dead cells unable to be amplified by PCR. MycoLight™ vPCR Star-based PCR has been shown to differentiate between non-viable and viable bacteria. It has been used to overcome the low selectivity in detecting bacteria and viruses based on its cell permeability. PMA is regularly used in vPCR detection of bacterial and viral samples. However, PMA's effectiveness requires strong UV irradiation of samples. UV irradiation is known to cause DNA damage and other detrimental effects to biological samples to be analyzed, thus resulting in inaccurate results (M. Kuick et al, Int J Mol Sci, 2020, 21(19), 7264; S. Kaur et al, Anal Chem, 2024, 2914-2921). In addition, UV irradiation requires operations carried out in the dark, which makes the sample analysis tedious and inconvenient with low throughput.

AT A GLANCE

Important Note

Before beginning the experiment, thaw MycoLight™ vPCR Star at room temperature and briefly centrifuge to collect the dried pellet.

Protocol Summary

1. Prepare bacterial samples.
2. Add MycoLight™ vPCR Star dye to bacterial samples.
3. Incubate samples for 10 to 20 minutes at RT.
4. Centrifuge samples to remove any excess dye.
5. Extract genomic DNA from bacterial samples.
6. Perform PCR/qPCR using appropriate primers and master mix.

PREPARATION OF STOCK SOLUTIONS

Unless otherwise noted, all unused stock solutions should be divided into single-use aliquots and stored at -20 °C after preparation. Avoid repeated freeze-thaw cycles

MycoLight™ vPCR Star Stock Solution

1. To prepare a 5 mM MycoLight™ vPCR Star stock solution, add 110 µL of DMSO to the vial of MycoLight™ vPCR Star.

SAMPLE EXPERIMENTAL PROTOCOL

1. Inoculate desired bacterial samples in a suitable media broth and culture them overnight, until the OD600 of the culture is near to 1.

   Note: This protocol is tailored for a 500 µL volume sample. Adjust the volume of the bacterial sample according to the experiment's scale.

2. **Optional:** To prepare dead cell controls, heat the bacterial sample (500 uL in a tube) at 90°C for 5 minutes.

3. Aliquot 500 µL of bacterial culture into individual clear microcentrifuge tubes. For each sample, prepare one tube for MycoLight™ vPCR Star treated cells and another for untreated cells (without MycoLight™ vPCR Star dye added) to calculate dCt values.

4. Add 5 µL of MycoLight™ vPCR Star stock solution to the bacterial samples.

   Note: Adding 5 µL of stock solution will result in a final dye concentration of 50 µM. This concentration can be used as a starting point and further optimization may be necessary to achieve optimal results.

5. Incubate samples for 10-20 minutes at room temperature.

   Note: The incubation time can be optimized for each experiment.

6. Centrifuge samples at 5000 x g for 10 minutes. Then remove the supernatant without disturbing the cell pellet.

7. Extract genomic DNA using your preferred method or a commercially available kit suitable for the sample type.

8. Perform qPCR using primers targeting a specific genomic DNA sequence of your choice.

   Note: Perform qPCR using the same volume for all samples. Normalization of the concentration of DNA is not required.

Data Analysis

1. After completing the qPCR, calculate the Ct (Threshold Cycle) value for each sample.

2. To determine if MycoLight™ vPCR Star has sufficiently inhibited the amplification of DNA in dead cells, calculate the delta Ct (dCt) for each of your control cells using the formulas below:
   - dCt (Live) = Ct (Live MycoLight™ vPCR Star treated sample) – Ct (Live untreated)
   - dCt (Dead) = Ct (Dead MycoLight™ vPCR Star treated sample) – Ct (Dead untreated)

3. For the live cell control, the expected result should be close to 0. A greater difference between dead and live cells suggests that the MycoLight™ vPCR Star treatment has effectively inhibited DNA in the dead cell samples.

EXAMPLE DATA ANALYSIS AND FIGURES

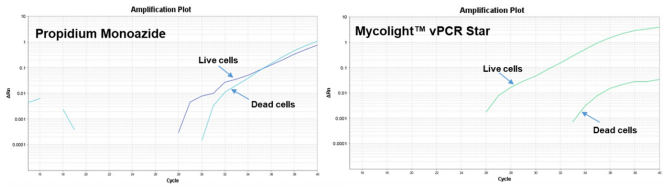

Figure 1. Normalized qPCR curves from a viability PCR experiment in which live and heat-inactivated E. coli were treated with MycoLight™ vPCR Star. qPCR was performed using primers against a region of the uidA gene. MycoLight™ vPCR Star treatment had no effect on amplification of DNA from live E. coli, but caused a significant delay in amplification of DNA from heat-killed E. coli. Propidium Monoazide did not work in the absence of light exposure.

DISCLAIMER

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